**Lab Nine: Oxygen and Spiking**

**OVERVIEW**

**In this lab you will:**

1. Learn about the importance of oxygen in generating neural action potentials;
2. Observe what happens to neural spikes when the system is deprived of oxygen; and
3. Test how long neurons can be “starved” of oxygen but still recover, and at what point the oxygen starvation is irreversible.

**OBJECTIVES**

**Before doing this lab you should:**

* review what you learned in Experiment 6 about neuron energy requirements for generating action potentials;
* study what it means for an organism to be “aerobic”, and why oxygen is necessary for their survival;
* learn some of the basics of how biochemical equations describe events that are important for life.

**After doing this lab you should be able to:**

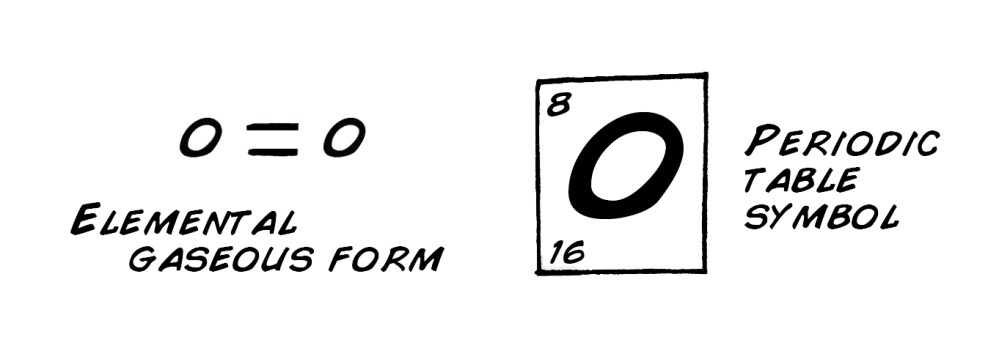
* explain why neurons stop generating spikes in the absence of oxygen, and what happens when oxygen is returned again;
* describe whether the “time it takes for the spikes to go away” in the absence of oxygen and the “time it takes for the spikes to come back” once oxygen returns is the same or different;
* design an experiment to test whether a different coating (besides petroleum jelly) can prevent the cockroach leg from drying out while still allowing oxygen to pass through.

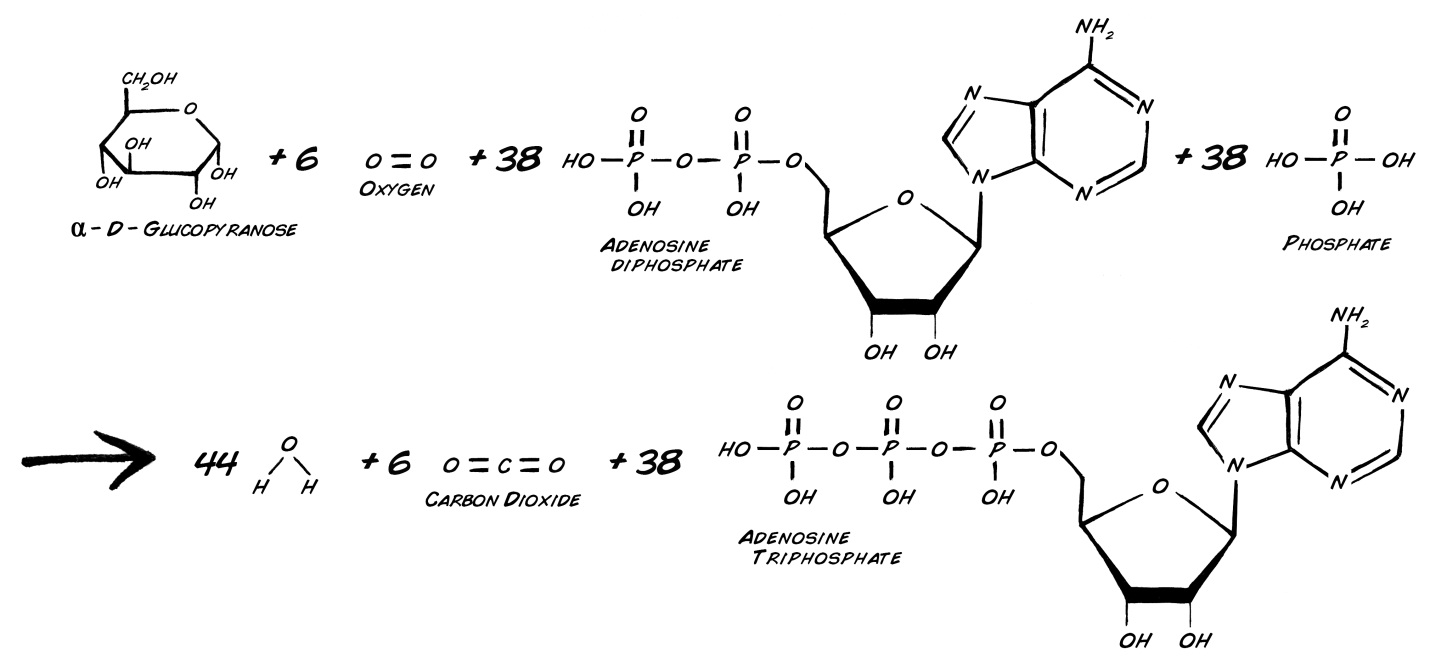
**EQUIPMENT**

* One SpikerBox (though you will find this experiment easier with two)
* Computer with Audacity installed or iPhone/iPad/Android with Backyard Brains app
* Associated laptop cable or iPhone/Android cable
* Cockroach (two cockroach legs)
* Two recording electrodes
* Dissection scissors
* Toothpick or skewer that is broken in half so one end is pointy
* Petroleum Jelly
* Stopwatch

**INTRODUCTION**

In Experiment 6 we talked about the energy that neurons need to continue generating spikes and specifically about the energy needed to operate the sodium-potassium pump. This energy is provided by adenosine triphosphate (ATP), which is made largely from the foods that you eat. Food, though, is only one of the things that your body requires to stay alive. What is invisible, tasteless, void of form, but yet critical to your life?

Oxygen! Oxygen is an “oxidizer”, meaning it is greedy for electrons. Pure gaseous elemental oxygen is so electron greedy that it was a poison for most life on Earth billions of years ago. In fact, the only elements greedier for electrons are fluorine and chlorine, both of which are still very poisonous in elemental gas form. Once life forms evolved to harness the oxidizing nature of oxygen, though, aerobic (oxygen breathing) creatures became the dominant organisms on the planet.

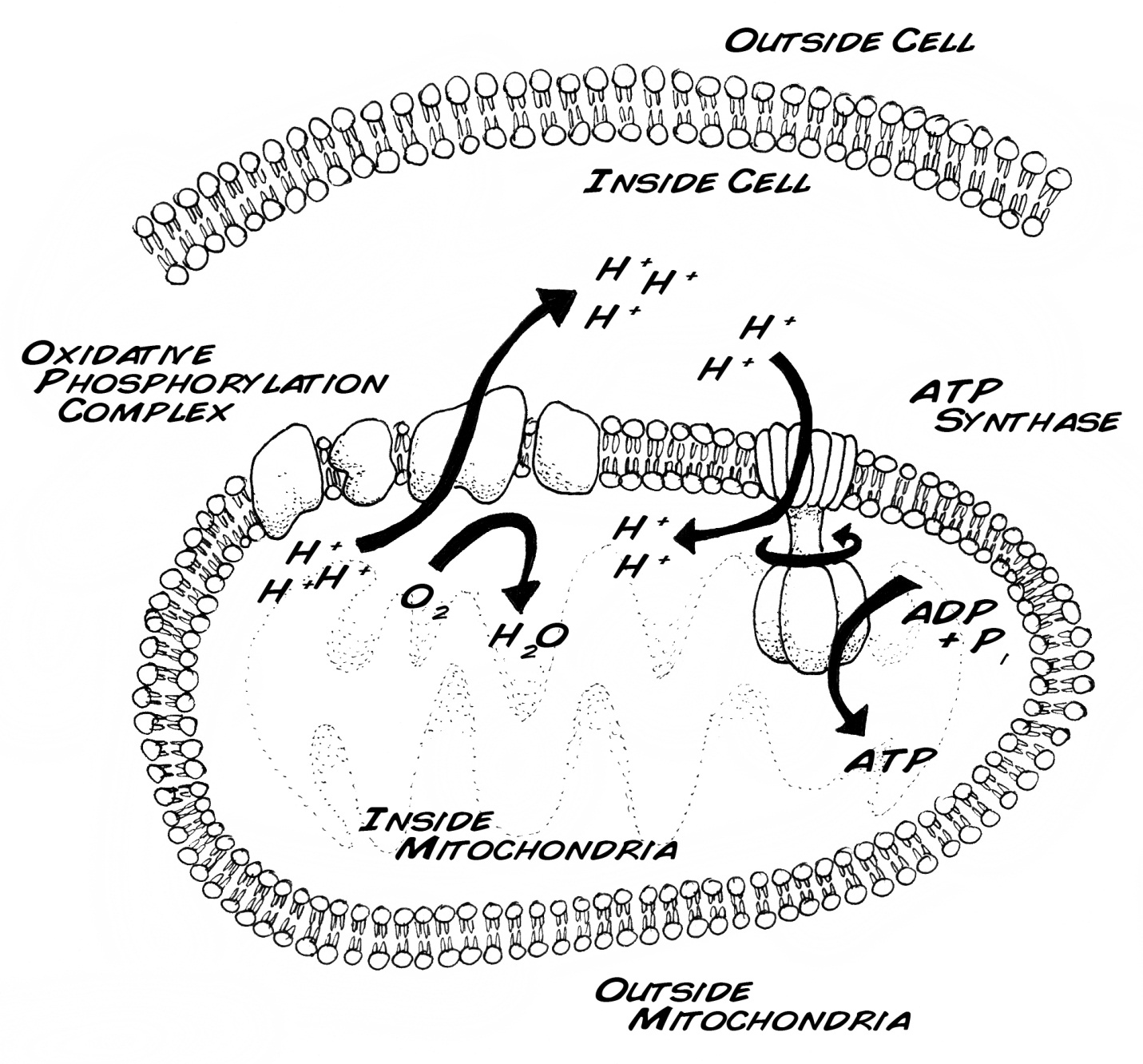
All animals, including insects, fish, birds, dogs, and you, need oxygen in addition to food to generate enough ATP to stay alive. The reasons and mechanisms for why oxygen is so vital to energy generation can be explained using biochemistry.

This complicated chemical reaction has dozens of tightly controlled steps that are classified into three different pathways:

1. Glycolysis
2. The Krebs cycle
3. The oxidative phosphorylation pathway

Glycolysis is the breakdown of the 6 carbon sugar into 3 carbon pyruvate. The Krebs (citric acid) cycle generates electron-rich molecules with funny names like NADH and FADH. The oxidative phosphorylation pathway is where the electrons from NADH and FADH are transferred through multiple molecules (with more funny names like coenzyme Q and complex IV) before going to the final electron acceptor, oxygen.

Oxygen is ultimately converted to H2O (water). As these electrons (which began with NADH and FADH) move through successive owners (like coenzyme Q, complex IV, and oxygen) in the oxidative phosphorylation pathway, this creates energy that is used to generate an excess of hydrogen ions (protons) outside the membrane of the mitochondria of the cell. The mitochondrion is the cell organelle where this oxidative phosphorylation pathway occurs. The excess protons outside of the membrane produce an ionic imbalance, which is used to power a very special enzyme: ATP synthase. The excess hydrogen ions then travel back into the mitochondria by going through the ATP synthase, which causes the synthase to “rotate”, which generates the work that converts ADP into ATP (through protein chemistry). This process is illustrated in the figure below.

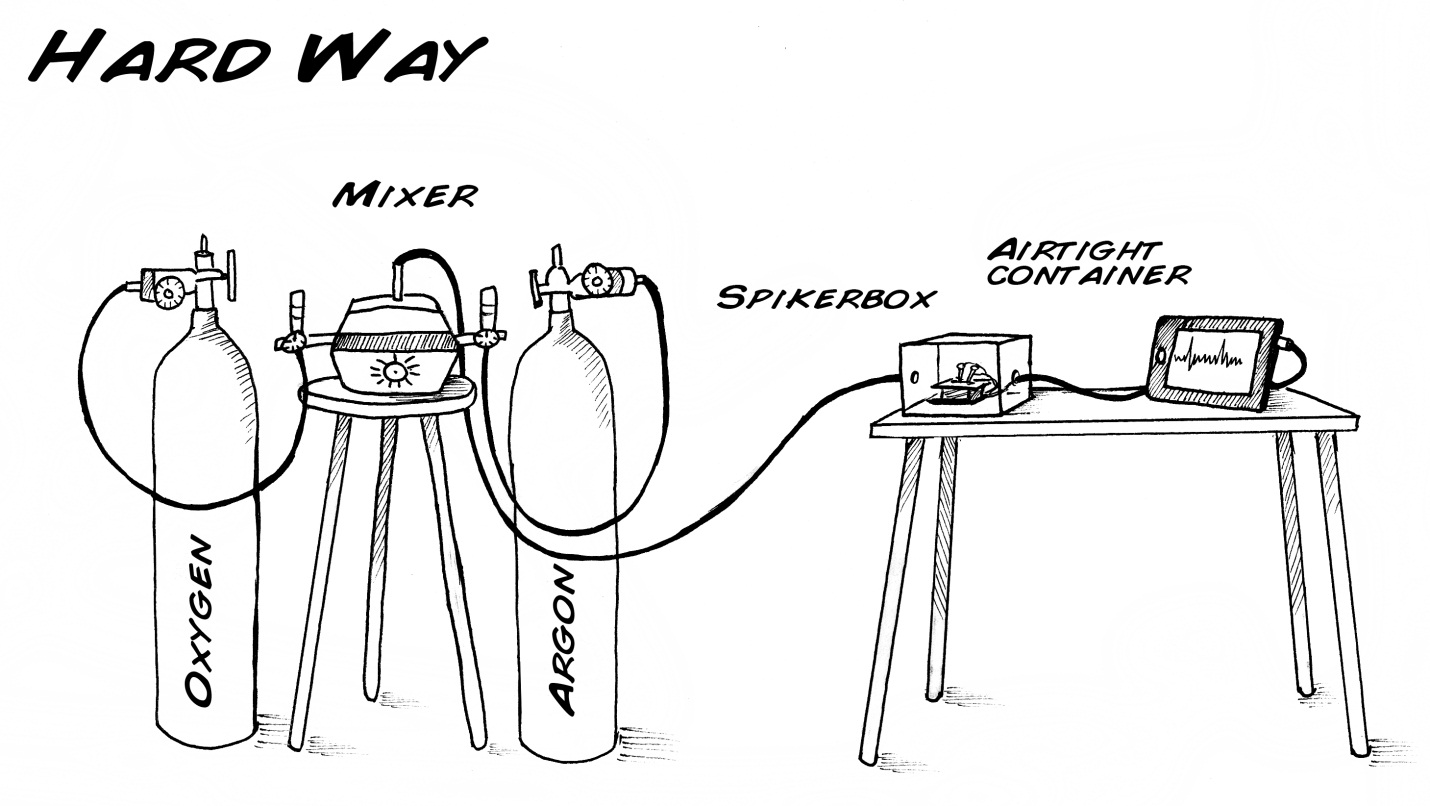


Take home message:

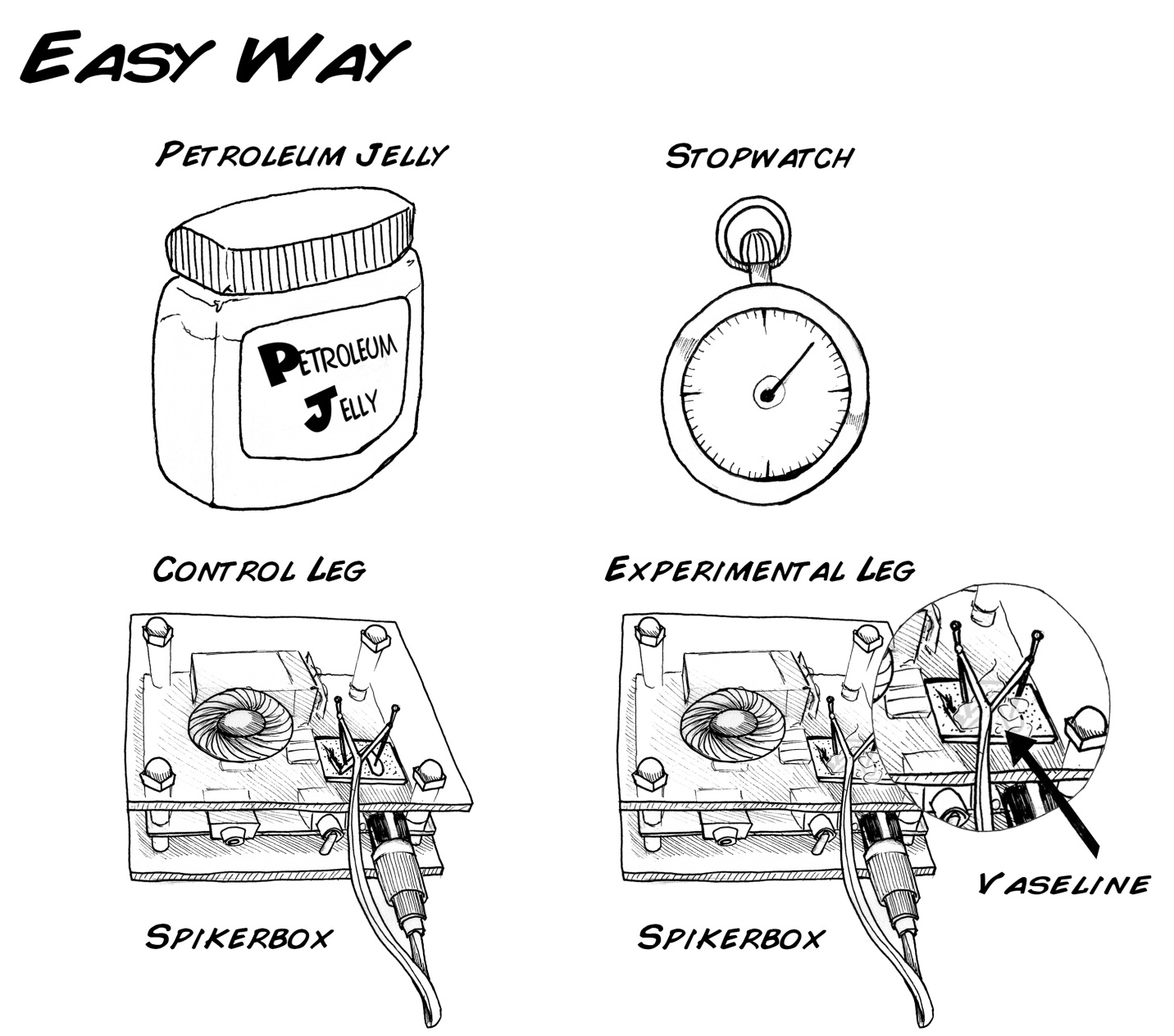
1. These three reaction pathways are necessary to break down the sugar into a form that the body can use
2. Two of the processes require oxygen in order to work (glycolysis doesn’t require oxygen)
3. One of the main outputs of these chemical interactions is ATP
4. ATP, as we learned in experiment 6, is the key energy generator required for neurons to maintain ionic balance across their membranes

Thus, without oxygen there is not enough energy for the body.

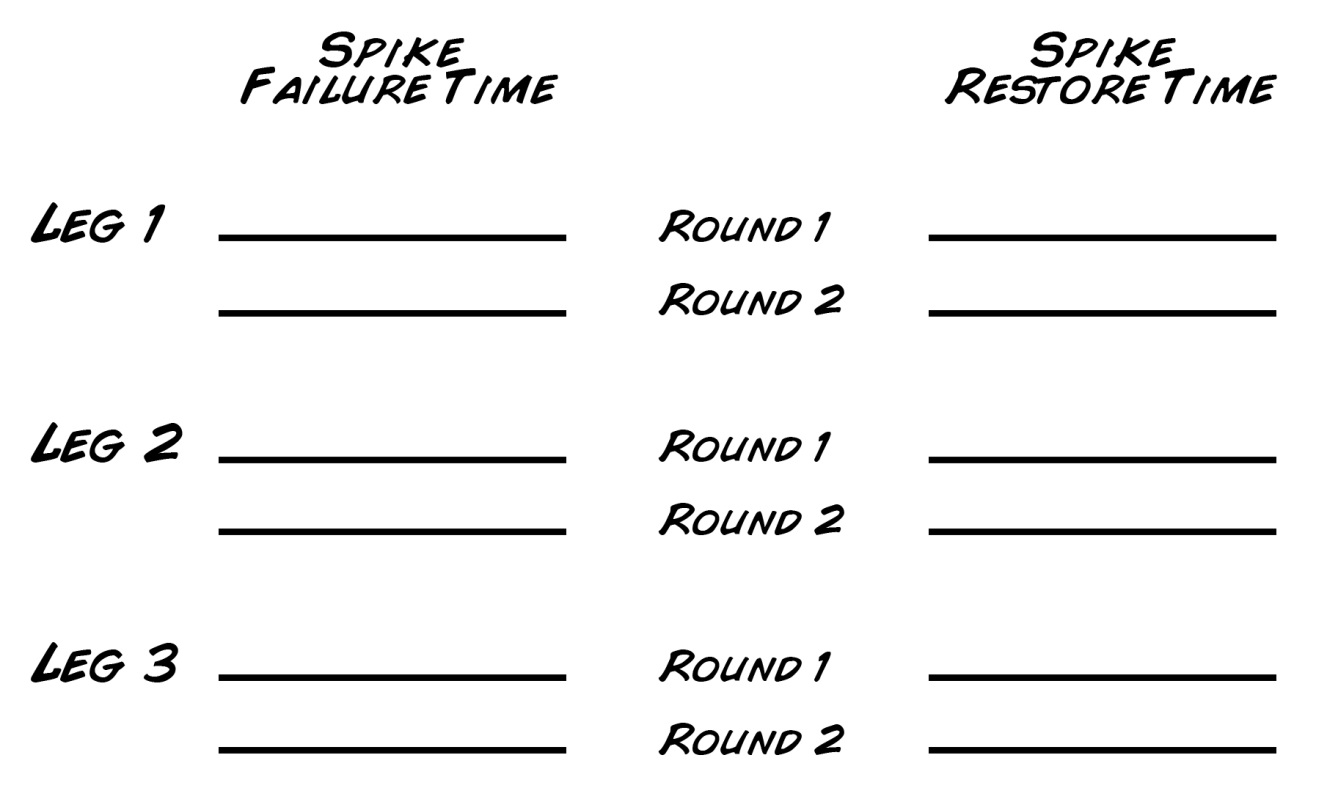
Now, let’s think back to how this applies to the cockroach legs that we record from. While the disconnected leg has enough food stored inside to survive for up to a couple of days (as we saw in Experiment 6), the leg does not have enough oxygen to survive for nearly as long. This same holds true for you…think how long you can go without eating, then compare that with how long you could survive without breathing. Big difference, huh? So, let’s see if we can investigate more specifically what the relationship is between oxygen and spike generation in the cockroach leg. And since we’ve been doing experiments for a while now, let’s take this opportunity to compare different ways to experimentally test the same concept and use our judgment on which approach makes the most sense for us to use.

One way to test this experimentally is to put your cockroach leg and SpikerBox in a positive airflow container. To do this, you would need to buy an oxygen tank and an argon tank (argon is typically used as an inert gas and would be the gas that we replace oxygen with in this experiment). You would also need some flow regulators and tubing, and you would then have to hook up the tanks such that you can slowly decrease the percentage of oxygen in the airtight container to see the effect that different levels of oxygen has on spiking activity in the cockroach leg. This experiment would give us a very detailed relationship between oxygen and spike generation but would be expensive and difficult to do, especially outside of a formal lab setting. So, let’s call this proposed experiment “the hard way”.

Thankfully, there is an alternative approach we can take. Since the leg of the cockroach gets all of its oxygen through the open wound (we discovered this when [we visited our friendly local electron microscopist](http://news.backyardbrains.com/2010/11/byb-visits-a-scanning-electron-microscope/)), we can “starve” the leg of oxygen by simply liberally coating the leg with petroleum jelly (vaseline).We’ll call this approach “the easy way” to do it, and continue with that approach for the procedure of this experiment.



**PROCEDURE**

1. Set up your computer and prepare cockroach legs as described in Experiment 1. Cut off two legs for this experiment. (Note: you can use two SpikerBoxes (one for each leg), but you don’t have to. It is possible to do this experiment with only one SpikerBox; you could simply swap out the electrodes that are hooked up to each cockroach leg)
2. Liberally coat one leg (the “experimental leg”) with petroleum jelly using a toothpick, and start your stopwatch. The “control leg” is the other leg and the one with no petroleum jelly on it.
3. Every 10 – 15 minutes, manipulate both the control and the experimental leg by touching the barbs with a toothpick to see if you can evoke spikes.
4. After a time, the experimental leg should stop spiking. Mark down the time that this occurs on the chart. (Note: if the experimental leg doesn’t stop spiking, you may not have put enough petroleum jelly on the leg to create a good seal against the air. If this happens, put on more jelly and re-start your stopwatch).
5. Carefully remove the petroleum jelly with a napkin or paper towel. Restart your stopwatch. Every 10 – 15 minutes, touch the barbs of both legs to attempt to evoke spikes. Eventually, the spikes should return to the experimental leg. Mark the time at which the spikes return on the chart above.
6. Repeat steps 2 – 5 as long as you have time for AND as long as the control leg is still generating spikes.
7. Repeat the experiment over a couple of legs to see if you can find an “average time for the spikes to go away” and an “average time for the spikes to come back”.

**DISCUSSION QUESTIONS**

1. It’s easy to understand why the spikes eventually go away when you starve the leg of oxygen, but why do the spikes come back? How long can the leg be coated in petroleum jelly before he spikes go away permanently?

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1. When doing this experiment, you have to monitor the control leg to ensure that it continues to generate spikes during the entire experiment. If the control leg stops generating spikes, you have to stop the experiment and/or move on to a new set of legs. Why is this control monitoring so important, and why do you have to stop the experiment once it stops generating spikes?

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1. After repeating this experiment a few times, you can find an “average time for the spikes to go away” and an “average time for the spikes to come back”. Are they different or the same? Why?

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1. You’ve probably figured out that the leg eventually dies from drying out. Is there a way you can keep the leg moist, while also allowing oxygen to pass through? Is there any material that is permeable to air but not water?

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